Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Document heading doi:10.12980/JCLM.2.201414B57

Antibacterial activity of essential oil of north west Algerian *Eucalyptus camaldulensis* against *Escherichia coli* and *Staphylococcus aureus*

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PEER REVIEW

Peer reviewer

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Comments

This is a valuable research work in which authors have demonstrated the antimicrobial activity of essential oil of *E. camaldulensis* against *S. aureus* and *E. coli*. The activity was assessed *in vitro* based on two methods of antimicrobial evaluation (dilution broth and disc diffusion techniques). *E. caemaldulnsis* oil was found to be a promising antimicrobial agent in treatment of the infections caused by these two germs.
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ABSTRACT

Objective: To evaluate the *in vitro* antimicrobial activities of the crude oil of *Eucalyptus camaldulensis* (*E. camaldulensis*) leaves.
Methods: The essential oils of *E. camaldulensis* harvested from the garden of the Health Center in Sidi Bel Abbes city (North West of Algeria), were screened for their antibacterial activities against two clinical bacteria [*Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*)] by the agar disc diffusion method and broth dilution susceptibility assay.
Results: The diameter of zones of inhibition by the leaf extracts of *E. camaldulensis* was 10-31 mm and 10-26 mm respectively for *E. coli* and *S. aureus*. Gram positive *S. aureus* was more resistant to tested essential oil than Gram negative *E. coli*.
Conclusions: The results suggested a potential antimicrobial activity of the essential oil of *E. camaldulensis*, which may find its application in future research for the food and pharmaceutical industry.

KEYWORDS

Eucalyptus camaldulensis, Essential oils, Antibacterial activity, *Escherichia coli*, *Staphylococcus aureus*

1. Introduction

Eucalyptus is one of the diverse genus of flowering plants in the world belongs to the family Myrtaceae (subfamily Myrtoideae) and comprises about 800 species[1]. *Eucalyptus* has been used in folk medicine throughout the world as anti-inflammatory, analgesic and antipyretic remedies for the symptoms of respiratory infections, such as cold, flu, and sinus congestion[2,3]. Essential oils from *Eucalyptus* species have been approved as food additives, and the extracts are also widely used in modern pharmaceutical, and cosmetic industries[4]. In addition, the oil possesses a wide spectrum of biological activity including anti-microbial, fungicidal, insecticidal/insect repellent, herbicidal, acaricidal and nematicidal[5]. The main uses of the leaves of some species are the production of essential oil[3]. In this work, the antibacterial property of the *Eucalyptus camaldulensis* (*E. camaldulensis*) leaf

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Foundation project: Supported by Project (F03720120003) of Ministry of Higher Education & Scientific Research in Algeria.

Article history:
Received 10 Apr 2014
Received in revised form 15 Apr, 2nd revised form 25 Apr, 3rd revised form 28 Apr 2014
Accepted 3 Jun 2014
Available online 22 Sep 2014

oils was checked against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*), which aims to the valorize of medicinal and aromatic plants of the Algerian flora and to find natural antibiotic to these two germs which have acquired drug resistance and cause severe diseases potentially life threatening conditions. The Gram positive bacterium *S. aureus* is mainly responsible for post operative wound infection, toxic shock syndrome and food poisoning.

E. coli, which is one of the Gram negative bacteria, is present in human intestines and causes urinary tract infection, coleocystitis or septicemia[6].

2. Materials and methods

2.1. Plant materials

The fresh leaves (matured) of *E. camaldulensis* were collected during the months of May and June 2010 (flowering season) from the garden of the Health Center which is located in Gambetta district, Sidi Bel Abbes city, northwest of Algeria, identified and authenticated at Environmental Sciences Department, Djillali liabes University of Sidi Bel Abbes.

2.2. Essential oil distillation

The fresh leaves were submitted to Hydro-distillation with a Clevenger-type apparatus. A total of 50 g of dried plant material and 300 mL of distilled water were used, and the distillation was carried out for 2 h after the mixture had reached boiling (until a clear distillate was obtained). The oil layers had separated from the water layers in a rotary evaporator to giving a light yellow extract.

The essential oil was obtained in a yield of 0.84% (w/w). The oil was stored in an amber flask in a refrigerator at 4 °C until required.

2.3. Bacterial strains used

E. coli and *S. aureus* clinically isolated from specimens of different infectious disease obtained from the Medical Analysis Laboratory, Dr. Hassani Abdelkader Hospital University Center of Sidi Bel Abbes City.

The isolates were identified on the basis of Gram's staining, motility, cultural characterization and biochemical screening-routine methods were used[7]. Hektoen enteric agar and Chapman medium (Mannitol Salt Agar) were prepared for isolation of bacteria.

2.4. Antibacterial screening

Antibacterial activity of *E. camaldulensis* leaf essential oil was tested against the above Gram-positive and Gram-negative bacteria by disc diffusion method and dilution broth technique. These bacteria were grown in Mueller-Hinton Agar medium (Pasteur Institute-Algiers) poured into sterilized Petri dishes with an uniform depth.

2.4.1. The disc diffusion method

The agar diffusion method is the most widespread technique of antimicrobial activity assessment. Disc-assay was found to be a simple, cheap and reproducible practical method. For this bioassay method, a suspension of each sample tested micro-organism diluted prior to 10^{-1}, 10^{-2} and 10^{-3} (1 mL of 10^{6} cells/mL) was spread on the Mueller Hinton Agar plates. About 6 mm diameter discs were prepared with Whatman paper and used for the study. About 10 μL of essential oil dilution (25%, 50%, 75%, 100%) was impregnated on the sterile filter paper discs, and placed on the surface of the plates. A total of 10 μL of ethanol was added to sterile filter paper disc as control. All the plates were incubated at 37 °C for 24 h. The entire microbial assay was carried out under strict aseptic conditions. After the completion of incubation period, the zone of inhibition produced by the sample with two tested organisms in different plates was measured in millimeters and recorded immediately[8].

2.4.2. The dilution broth technique

Dilution broth susceptibility assay was used for screening the antimicrobial activity of the oils against *S. aureus* and *E. coli*[9]. Stock solutions of the essential oils were prepared by dissolving 1 mL of the extracts with 9 mL of alcohol in test tubes to obtain the mother solution, from which we prepare our samples by means of successive dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}). A control was prepared similarly using equal amount of sterile distilled water (1 mL) in place of the essential oil. A total of 1 mL of each dilution and 0.5 mL of tested culture strains were introduced in sterile test tubes and nutrient broth (8 mL) added. The mixture was maintained in a Marie bath at 37 °C under stirring for 24 h. The contents of the positive tubes were streaked on the surface of the agar medium and incubated at 37 °C for 24 h. The antibacterial activity was assessed by the presence or absence of the culture.

3. Results

The antimicrobial activity of the crude aqueous extract of *E. camaldulensis* leaves was studied against two different clinically isolated strains of both Gram-positive and Gram negative bacteria. The potency of the essential oil was assayed by the presence or absence of inhibition zones and zone diameter. The results of the agar disc diffusion assay are presented in Figures 1 and 2. They showed that the essential oil of *E. camaldulensis* has significant antibacterial activity against the two bacterial strains tested. The zone of inhibition of the two bacterial strains tested ranged between 10 mm and 31 mm. Inhibition zones diameters for *S. aureus* showed a variation which ranged between 10 mm and 26 mm. The zones of inhibition are comparable with those of the

control.

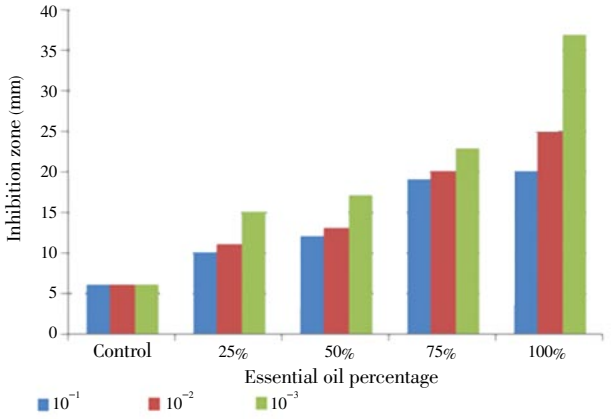

Figure 1. Sensitivity patterns of zones of inhibition exhibited by *E. camaldulensis* crude aqueous leaf extract against *E. coli*.

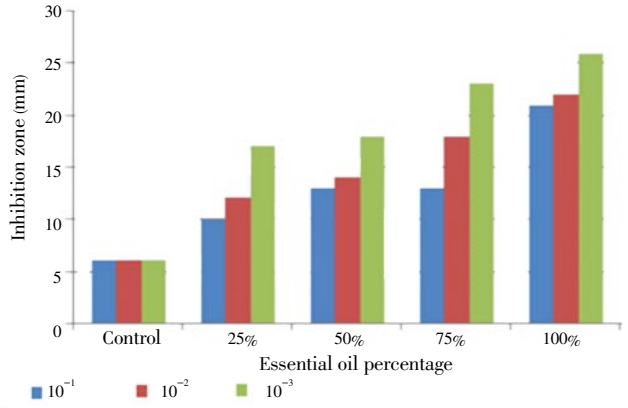

Figure 2. Sensitivity patterns of zones of inhibition exhibited by *E. camaldulensis* crude aqueous leaf extract against *S. aureus*.

The highest zone of inhibition was obtained for *E. coli* (10^{-3} dilution) with 100% concentration of essential oil of *E. camaldulensis*, while the lowest activity was shown against the same germ (10^{-1} dilution) with 25% concentration of the crude extract.

With increase in concentration of essential oil, increase in zone of inhibition was observed. Higher extract concentration (100%) exhibited higher activity against the microorganisms used, in comparing with the lower sample concentration (25%).

In contrast, the results of the dilution broth method shown in Table 1 were in agreement with the results of previous method.

Table 1

Antimicrobial activity evaluation of the essential oil of *E. camaldulensis* leaves using the dilution broth method against the two bacterial strains.

Microbial strains	Essential oil dilution					
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Control
E. coli	+	+	++	++	+++	+++
S. aureus	+	+	+	++	+++	+++

+++: Important growth of organisms; ++: Moderate growth of organisms; +: Slow growth of organisms.

As seen the studied essential oil demonstrated antimicrobial activity against the two tested microorganisms, except the dilution 10^{-3} where we see that the Gram-positive bacteria (*S. aureus*) was found to be more susceptible to this extract than *E. coli*, a Gram-negative model. The essential oil exhibited concentration-dependent inhibition of growth. The crude aqueous leaf extract was active against *S. aureus* and *E. coli* at high concentrations (10^{-1}, 10^{-2}) when compared to the low doses (10^{-5}).

4. Discussion

The antibacterial activity of essential oil of *E. camaldulensis* was evaluated by disc diffusion method and dilution broth technique against two bacterial clinical isolates. According to the given results, *Eucalyptus* extract exhibited an interesting antimicrobial activity against the tested microorganisms. It is evident from the results that the antimicrobial activities increase when increasing the oil concentration from 25% to 100%. During disc diffusion method, the results indicated that the essential oil of *E. camaldulensis* had more inhibitory effects on Gram-negative bacteria than Gram-positive bacteria.

In the most literature, Gram-negative bacteria were more resistant to essential oils than Gram-positive bacteria[10]. However, not all studies on essential oils conclude that Gram-positive bacteria are more susceptible to essential oil[11]. Many researches demonstrate that Gram-positive bacteria have been found to be less or equally sensitive comparing with Gram-negative bacteria as well[12-16]. Dorman and Deans showed that antibacterial activity depends on the type of essential oil[17]. Kim *et al.* suggested that the antibacterial activity does not depend on the type of Gram reaction[18]. Our results shown that *S. aureus* Gram-positive strain was more resistant than *E. coli* Gram-positive bacteria model.

In dilution broth assay, no clear differences in the level of susceptibility between Gram-negative and Gram-positive bacteria were observed. To some extent, these results were similar to those of previous studies. Mishra and Mishra[16], who studied the effect of *Ocimum sanctum* essential oil against *E. coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *S. aureus*, concluded that Gram-positive and Gram-negative organism were equally susceptible to the antimicrobial action of extracts. Dorman and Deans reported that the volatile oils of *Origanum vulgare* subsp. *hirtum*, *Piper nigrum*, *Syzygium aromaticum* and *Myristica fragrans* did appear to be equally effective against both Gram-positive and Gram-negative microorganisms[17].

The permeability of the bacterial membrane, the presence of porin proteins in Gram-negative bacteria and the intracellular distribution of the oil constituents are key elements that influence the diffusion and the action of the essential oil into the cell. Therefore, further investigations will be required to understand the mechanism of

antimicrobial action of essential oils as a mixture of numerous molecules[19].

Most previous studies of *Eucalyptus* antibacterial activity have reported on the antimicrobial activity of oils with variable results. Cimanga *et al.* demonstrated the antibacterial activity of essential oil extracted from *E. camaldulensis* leaves against *E. coli* and *S. aureus* with zone diameter of inhibition 10-12 mm and 18-30 mm respectively[20]. Trivedi and Hotchandani showed that strains of *E. coli*, and *S. aureus* were inhibited by the commercially available *Eucalyptus* oil[21]. *E. coli* was sensitive to 5 mL while *S. aureus* required 25 mL of the extract. Akin-Osanaiye *et al.* reported complete inhibition of *S. aureus* and a production of 80.5 mm zone diameter of inhibition for *E. coli* in the application of essential oil of *E. camaldulensis* collected from Kadune state in Nigeria[22]. Oskay and Sarı showed that *E. camaldulensis* oil from Manisa province (Turkey) possessed a significant activity against the Gram-negative bacteria *E. coli* and Gram-positive bacteria *S. aureus* (10 and 18 mm, respectively)[23]. Ayepola and Adeniyi reported that the methanolic extract of *E. camaldulensis* leaves collected from Ibadan city (Nigeria) was most active compared to dichloromethane fraction against *S. aureus* with diameter of zone of inhibition as 15 and 13 mm respectively[24]. Similarly, the results in Nezhad *et al.* study showed that alcoholic extract of *Eucalyptus* collected in September 2008[25], in Tehran (Iran) is higher against SAMR (ATCC 25923) than aquatic extract. In *mecA* negative *S. aureus* the maximum zone of alcoholic extract was 18 mm at 30 μL volume whereas in *mecA* positive *S. aureus* the maximum zone of alcoholic extract was 14 mm at 30 μL volume. In addition, Owliaa *et al.*[26] reported that *E. camaldulensis* oils have properties that can inhibit the growth of *Pseudomonas aeruginosa* Gram-negative bacteria like *E. coli*- with 12 mm diameter of zone of inhibition. Oskay *et al.*[27], screened the ethanolic extracts of 19 plant species against 10 clinical isolates and reported that *E. coli* and *S. aureus* were moderately susceptible to *E. camaldulensis* oil with their respective diameter zones of inhibition of 16 and 18 mm. Recently, Akin *et al.* tested *in vitro* antibacterial activity of *E. camaldulensis* Dehn. and *Myrtus communis* L., collected from Northern Cyprus against seven bacteria[28]. Of the tested oils, *E. camaldulensis* was found to be most active against *S. aureus*, showing inhibition zone of 14 mm in diameter but no inhibition of the growth was seen in *E. coli*. The zone of inhibition (26 mm diameter) due to the essential oil vapours of *E. camaldulensis* collected from the Dezfoul city (southwest of Iran) against *S. aureus* ATCC 25923 was recently reported by Panahi *et al*[29]. In other study, Abubakar reported that the least activity in terms of zones of growth inhibition was shown by *E. camaldulensis* aqueous extract against *E. coli* (7 mm)[30], and *S. aureus* (12 mm) while the highest was demonstrated by the acetone extract with a recorded zone diameter for *E. coli* (12 mm), and *S. aureus* (14 mm).

A large number of studies have reported that the essential oils of *Eucalyptus* species are the most potent regarding their antimicrobial properties[3,8,22,31-33].

The antimicrobial activity of the essential oil from *Eucalyptus* leaves can be attributed to the presence of high concentration of 1, 8-cineole (15%-78%)[34], which has been reported to stimulate respiration, relieve coughing, helps to expel mucus, relax the respiratory muscles, and thus it is used for the management of bronchitis, asthma, catarrh, sinusitis and throat infections[35]. It has been found to have relatively strong antimicrobial properties against many important pathogens and spoilage organisms including *S. aureus* and *E. coli*[36]. In addition, other compounds such as α-Pinene, p-cymene, β-caryophyllene, β-pinene, spathulenol and carvacrol, which have relatively strong antibacterial properties may be responsible for this activity[17,28,37-39]. The minor compounds such as borneol, pulegone, thujone, γ-terpinene and nerolidol already are known to exhibit an antibacterial activity[28,40-45]. Therefore, the synergistic effects of these active chemicals with other constituents of the essential oil should be taken into consideration for the antimicrobial activity[19,36,40].

In conclusion, the crude extract of *E. camaldulensis* leaf was found to have significant antibacterial activity against the two tested strains and thus confirmed traditional medicine use of *Eucalyptus* oil as an antibacterial agent. The results of this study therefore form a good basis for selection of *E. camaldulensis* oil for further *in vivo* studies and clinical trials for their use as a natural antimicrobial agent for the treatment of several infectious diseases caused by these germs, which have developed resistance to antibiotics.

Acknowledgement

This work was supported by Project (F03720120003) of Ministry of Higher Education and Scientific Research in Algeria.

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

The Gram-positive bacterium *S. aureus* is mainly responsible for post operative wound infection, toxic shock syndrome and food poisoning. *E. coli*, which is one of the

Gram-negative bacteria, is present in human intestines and causes urinary tract infection, coleocystitis or septicemia. However, both germs exhibited multiple resistances to antimicrobial drugs and high prevalence of the antibiotics resistance.

Research frontiers

The present study research aims to find a solution for the antibiotic resistance. The essential oils are extracted from the fresh leaves of *E. camaldulensis* collected at the garden of Sidi Bel Abbes city of Algeria. Its activities were tested against two microorganisms, *S. aureus*, and *E. coli* clinically isolated from specimens of different infectious disease.

Related reports

Previous study demonstrated the antibacterial activity of essential oil extracted from *E. camaldulensis* leaves against *E. coli* and *S. aureus*. Another study also showed that strains of *E. coli*, and *S. aureus* were inhibited by the commercially available *Eucalyptus* oil. The antibacterial activities are suspected to be associated with the high contents of oxygenated terpenes components.

Innovations and breakthroughs

Pine is widely used in traditional therapeutic practice in world. It have been used against respiratory diseases, coughs, colds and rheumatic pains. In the present study, authors have demonstrated that the *E. camaldulensis* leaf essential oils possess antibacterial activities against these two bacteria which are responsible for several infections.

Applications

From the literature survey it has been found that *E. camaldulensis* is safe to humans. This scientific study supports and suggests the use of the essential oil of this plant as antimicrobial agents in the search for new drugs.

Peer review

This is a valuable research work in which authors have demonstrated the antimicrobial activity of essential oil of *E. camaldulensis* against *S. aureus* and *E. coli*. The activity was assessed *in vitro* based on two methods of antimicrobial evaluation (dilution broth and disc diffusion techniques). *E. caemaldulnsis* oil was found to be a promising antimicrobial agent in treatment of the infections caused by these two germs.

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